Descriptive Morphology of Eggs of Some Species in the *Macrobiotus hufelandii* Group

(Tardigrada: Macrobiotidae)

ALBERT A. GRIGARICK, ROBERT O. SCHUSTER
AND ELIZABETH C. TOFTNER
Department of Entomology, University of California, Davis, 95616

The identification of species of *Macrobiotus* is frequently dependent more on characteristics of the egg than on features of the adults. The existing descriptive morphology of these eggs has resulted mainly from observations by light microscopy before the development of phase contrast and with scant attention being paid to details now recognized as important for species discrimination. Light microscopes can provide usable images only to 1,000× and can't resolve small but useful details. Phase systems provide additional information but still can't detect minute detail, distinguish between surface and subsurface features, or provide much information concerning surface topography. Vague and inconsistent terminology used to communicate information has lessened the value of light microscope observations. Illustrations are often of poor quality and written descriptions are usually incomplete and are not always compatible with the drawings.

The scanning electron microscope (SEM) provides capabilities for observing new information about these eggs and for photographically recording this information in a manner that can be communicated precisely. In addition, information so recorded can be easily quantified and analyzed by existing systems. We have developed a simple method for recording and analyzing information in a reproducible and communicable form and have attempted to identify this information with species of *Macrobiotus* in the hufelandii group.

PROCEDURE

To be able to study eggs of reasonably consistent quality, a standardized procedure was used for the preparation of all samples. The eggs, in small quantities of water, were fixed with boiling 95% ethanol and later dehydrated in absolute isopropanol. The isopropanol was replaced by amyl acetate and the specimens critical-point dried (Anderson, 1951) using CO_2 at 69 K cm^2. Silver conductive paint was used to affix the eggs to SEM stubs and the specimens were vacuum coated with 100 Å of silver followed by 100 Å of gold.

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Each egg was photographed using a Cambridge Stereoscan Mark II microscope. The photographs were taken at approximately 1,000 diameters and these images were used to determine egg diameter, the number of processes per hemisphere, the lengths and widths of processes and the amount of serration of the apical discs.

A Metals Research Instrument Corporation "Quantimet 720" Image Analyzing Computer (IAC) was used to detect and record features of the photographic enlargements of the egg shells. High contrast 3,000 diameter enlargements of each egg were examined using a 25 mm lens on the video scanner and the information was displayed as a format of 300 × 300 picture points (PP), with each PP representing a length of 0.13 mm of the 3,000× photograph. Expressed as micra, an area of $13\mu \times 13\mu$ (169 square micra) of egg surface was analyzed, and each PP represented a length of 0.043μ or an area of 0.0018 sq. micra.

The following characteristics of each egg were recorded with the IAC: 1) total pore area; 2) total number of pores; 3) pore size distribution expressed as the number of pores with diameters greater than 0.258μ, 0.344μ, 0.430μ, 0.516μ, 0.602μ, 0.688μ, and 0.774μ; 4) total perimeter of pores.

The hufelandii group of *Macrobiotus*, further limited to those for which the pharynx contains two macroplacoids but no microplacoid, contains the following species: *hufelandii* Schultze, *hufelandii recens* Cuénot, *pseudohufelandii* Iharos, *hibiscus* Barros, *anderssoni* Richters, and *inermis* Binda and Pilato. Fifty eggs of this group from worldwide localities were examined. The literature was surveyed for descriptive information and drawings of the eggs of these species. Our photographs of eggs best agreeing with published information were assumed to represent those species. New information provided by this study is presented 1) as photographs, and 2) as detailed description provided by SEM and IAC study.

Many of the eggs could not be assigned to described species because they differed from presently accepted parameters of size, number of processes, or sculpture of the shell. The application of numerical taxonomy programs to accurate measurements of multiple characters will eventually be used to delimit species and populations. The potential of a combined SEM-IAC approach to secure information suitable for statistical studies has been demonstrated by applying 2-way analysis of variance and a mean separation by Duncan's multiple range test for the small samples now available. For three species with multiple specimens the analysis of variance used only four replications of selected characters for the entire egg and three replications for characters of

TABLE 1. Mean values for measurements of eggs of *Macrobiotus* based on SEM and IAC data. Mean values of characters of different species associated with the same letter are not significantly different from each other at the ** 1%, or * 5% levels according to Duncan's multiple range test.

	nr. hibiscus n = 2	hibiscus n = 6	anderssoni n = 4	hufelandii n = 8	? hufelandii n = 1	hufelandii recens n = 1
ENTIRE EGG						
Egg diameter in μ**	70	58b	81a	73ab	82	53
Number of processes/ hemisphere**	143	117c	34a	81b	62	89
Process diameter in μ**	5	4b	10a	6b	8	5
Process length in μ**	6	5b	16a	7b	10	7
Discal serrations	9	9	10	16	4	10
Disc open or closed apically	closed	closed	closed	closed	open	closed
169 SQ. MICRA OF EGG SURFACE						
Number of pores**	41	142b	76ab	26a	0	168
Pore area in sq. μ*	19	33b	36b	13a	0	25
Pore perimeter in μ**	59	148b	109b	35a	0	121
Pore diameter distribution						
>0.258μ	40	146	71	24	0	137
>0.344μ	40	103	66	22	0	107
>0.430μ	38	81	60	22	0	62
>0.516μ	32	66	55	19	0	36
>0.602μ	25	52	46	15	0	16
>0.688μ	18	40	34	12	0	11
>0.774μ	14	28	30	9	0	7

the egg surface although the numbers of individuals indicated in table 1 are greater.

DISCUSSION

Macrobiotus hufelandii Schultze, 1833 (table 1, fig. 3)—the original description of *hufelandii* didn't include the egg stage. Doyère (1840) first described it as spherical and studded with processes swollen at the top but didn't mention whether the shell had pores. Most authors presently agree that the egg of *hufelandii* has a smooth shell with a circle of pores around the base of each process.

Eight eggs agreeing with this description were available from collections made in Sweden. These eggs are most distinguished by the reduced number of pores. The smallest pore area was 2 sq. μ per 13 μ sq. with pore diameters in the range of 0.43μ or less.

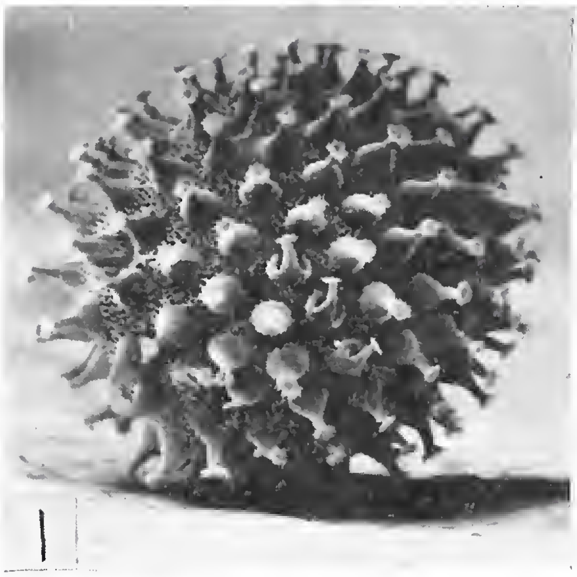
Macrobiotus sp. ? **hufelandii** (table 1, fig. 5)—a single example of an egg of *hufelandii* from California differs from the above examples in having a wrinkled shell and a total absence of pores. The processes are hollow with internal openings basally to each of the ridges radiating outward from the processes. The space between the inner and outer shell walls thus contains an appreciable amount of air space although the shell is not porous. If further study doesn't provide intergrades between the forms, the Californian population will need to be recognized as a distinct entity.

Macrobiotus hufelandii recens Cuénot, 1932 (table 1, fig. 1)—the egg of this species in no way resembles the illustration by Cuénot but agrees exactly with that illustrated by Ramazzotti (1972) as an egg probably representing *recens*. The former *recens* egg has about twice as many processes of lesser diameter and processes without distal discs. The variation is inordinate and the species represented in figure 1 may eventually be recognized as distinct. Of the kinds of eggs considered here, this one has the greatest total number of pores and largest pore perimeter.

Macrobiotus anderssoni Richters, 1907 (table 1, fig. 2)—examples of this species agree with the figure accompanying the original description. The small number of long processes with microdentate rings and the extremely porous shell surface is distinctive.

Macrobiotus hibiscus Barros, 1942 (table 1, fig. 4)—six eggs of *hibiscus* were from four widely separated localities. . . Tanzania, Italy, Finland and California. The eggs are small with many widely spaced small processes. The interprocess area is comprised of pores differing greatly in size.

Macrobiotus sp. nr. **hibiscus** (table 1, fig. 6)—a few eggs of a general *hibiscus* configuration appear to represent undescribed species. One example of these is a large egg with many closely spaced processes and few pores in the reduced shell area. Any understanding of the significance of these various eggs is dependent on the accumulation of sufficiently large numbers to allow a statistical analysis of their variation.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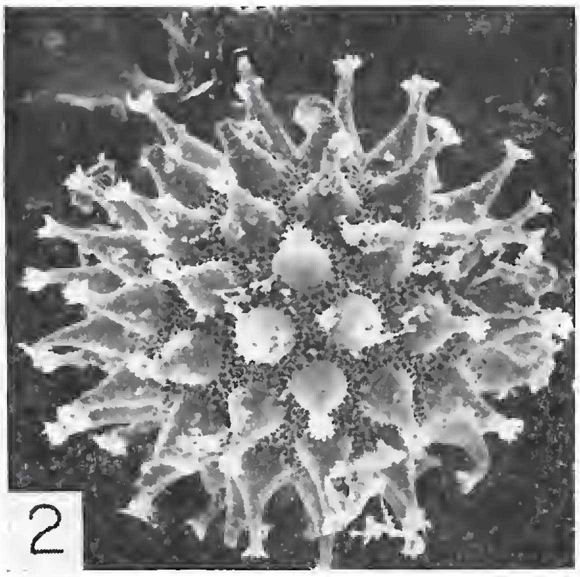
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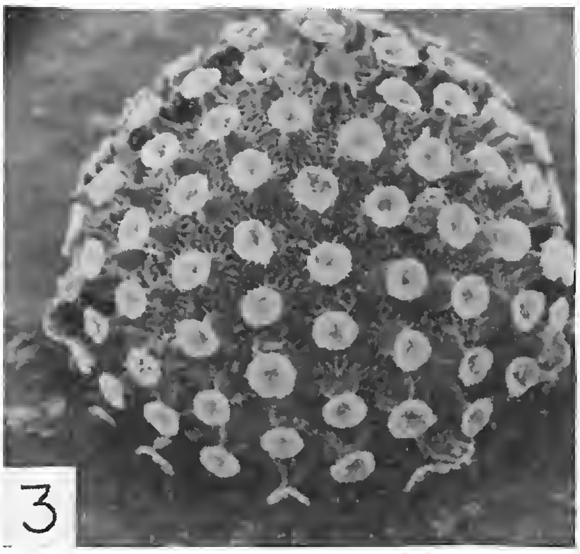
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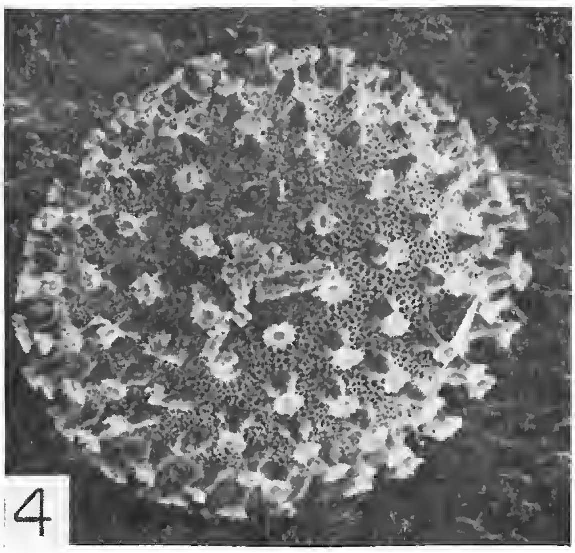
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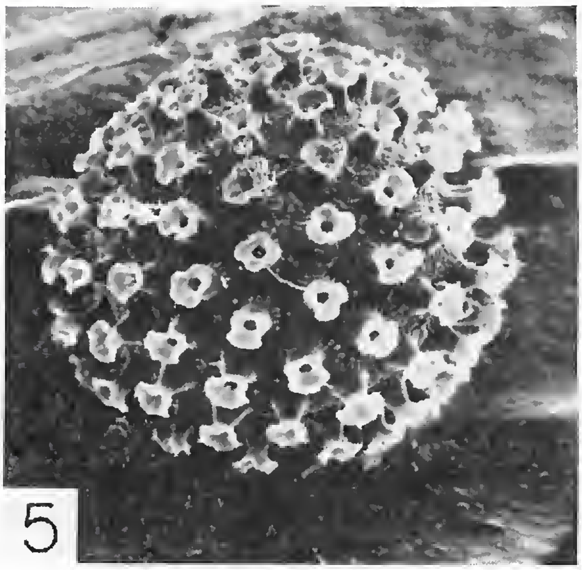
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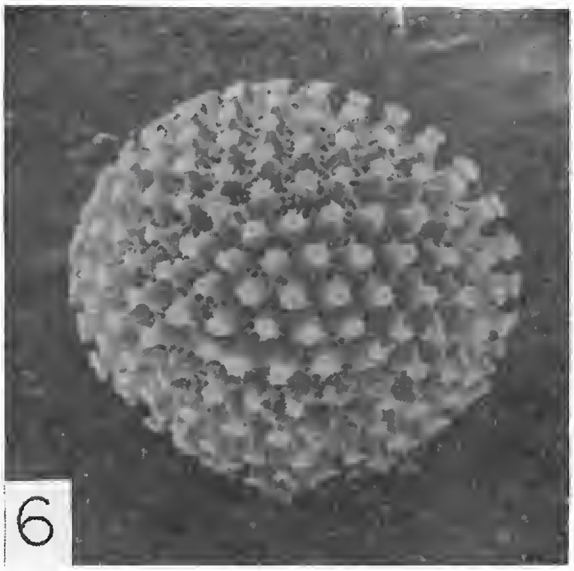
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Macrobiotus pseudohufelandii Iharos, 1966—we haven't seen an example of this egg which is described as having squat cone-shaped processes about 5μ high and 5μ wide at the base. The description indicates some 35–40 processes per hemisphere but the illustration has over 80. The shell is supposedly smooth.

Macrobiotus inermis Binda and Pilato, 1971—One extremely flattened egg was examined following removal from a slide preparation. The original description of this egg was essentially correct except the shell surface has about 45 minute pores in each area bordered by the bases of any four processes.

Closely spaced, short cone-shaped processes with small terminal discs have walls that are apparently smooth but a granular texture is evident at magnifications above 5,000×.

The eggs in *inermis* and *pseudohufelandii* appear to be extremely similar. Examination of eggs of the latter species by SEM will be required to resolve the question of possible synonymy.

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FIGS. 1–6. Scanning electron microscope photographs of eggs of *Macrobiotus* 1. *M. hufelandii recens* Cuénot, 700× (Mt. Aspiring National Park, South Island, New Zealand). 2. *M. anderssoni* Richters, 470× (Abel Tasman National Park, South Island, New Zealand). 3. *M. hufelandii* Schultze, 500× (Oland, Sweden). 4. *M. hibiscus* Barros, 675× (Muonio, Finland). 5. *M. ? hufelandii*, 455 (Auburn, California). 6. Undescribed sp. nr. *hibiscus* 500× (Triangulation Station, Chatham Islands, Rangatira Island, New Zealand).